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ANATOMICAL CONNECTIONS OF THE VENTRAL, BUT NOT THE DORSAL PART OF THE STRIATUM WITH THE PARVICELLULAR RETICULAR FORMATION: IMPLICATIONS FOR THE ANATOMICAL SUBSTRATE OF ORAL MOVEMENTS

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SUMMARY

Using a double labelling procedure by combining injections of the anterograde tracer *Phaseolus vulgaris* leucoagglutinin into different parts of the striatum with injections of the retrograde tracer choleratoxin subunit B into the parvicellular reticular formation of rats (pcRF), it was found that the ventrolateral part of the striatum (vlS) projects onto neurons in the dorsolateral part of the substantia nigra pars reticulata (SNR), a region that, in turn, was found to project to the pcRF. In contrast, the dorsomedial part of the striatum (dmS) projects onto neurons in the ventrolateral part of the SNR, a region that was found not to project to the pcRF. These differential striato–nigro–reticular pathways are discussed in view of the differential role of the vlS and dmS in the expression of oral movements.

KEY WORDS: striato–nigro–reticular pathways; parvicellular reticular formation; PHA–L; CTB; oral movements.

INTRODUCTION

It has been shown that neurons arising in the ventrolateral part of the striatum (vlS) primarily converge onto neurons in the dorsolateral part of the substantia nigra pars reticulata (dlSNR) which projects to the medullary reticular formation (RF), a region whose neurons are directly connected with orofacial motor nuclei such as the trigeminal motor nucleus (Vmo, 1–3). Based upon available literature, these pathways are suggested to serve as anatomical substrates for the expression of oral movements (1). Indeed, we (4,5) and others (6–8) have found that activation of dopamine D_1 and/or

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D_2 receptors in the vlS elicits jaw movements in the rat, showing that the vlS plays a critical role in the generation of oral movements. In contrast, neurons arising in the dorsomedial part of the striatum (dmS) primarily converge onto neurons in the ventrolateral part of the SNR (vlSNR; 9–11). It is unknown whether this vlSNR also projects to the RF. In this context, it is relevant to note that the dmS appears to play only a regulatory role in the expression of oral movements (4,5,12): it does certainly not play a critical role in the generation of these movements, since direct activation or blockade of dopamine D_1 and/or D_2 receptors in the dmS cannot elicit oral movements (4). These pharmacological data suggest that the vlS and the dmS are differentially connected with the RF. Given the extensive studies of von Krosigk et al. (1) on the vlS–dlSNR–RF connections, we primarily focused our attention on the possible dmS–vlSNR–RF connections. Thus, the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA–L; 13,14) was injected into the dmS or vlS, namely regions delineated previously in our pharmacological studies as regions that have a differential effect in the expression of oral movements (fig. 1; 4,5,12), and the retrograde tracer cholera toxin subunit B (CTB; 15) was injected into the RF. In comparison with von Krosigk et al. (1), we used CTB instead of horseradish peroxidase in order to limit the diffusion of the injections in the RF to the parvicellular part of the RF (pcRF), since that part contains neurons which are connected with Vmo (3).

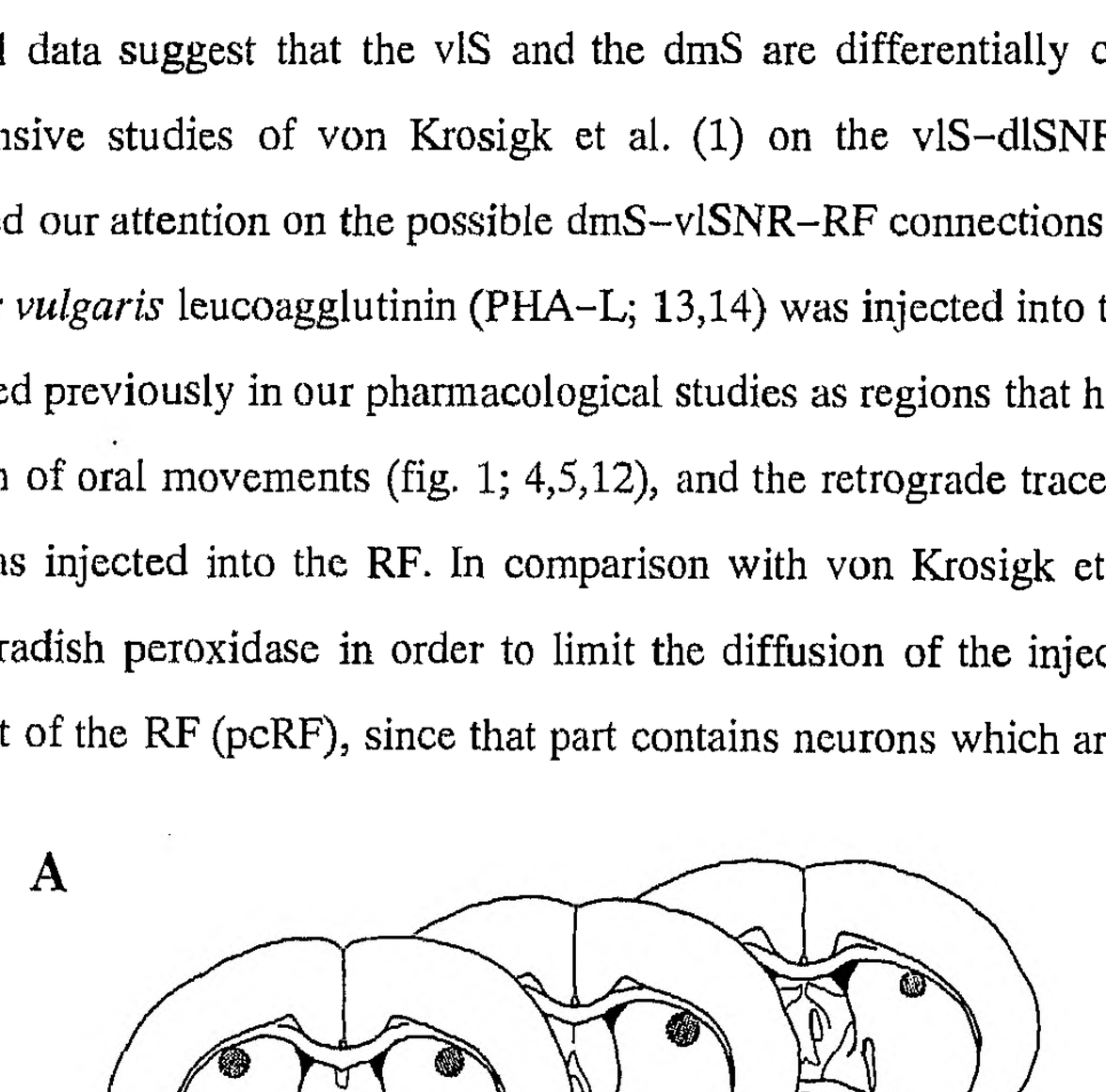

Fig. 1. Schematic drawing of the dorsal (A) and the ventral (B) regions in the striatum that have been found to have a differential role in the expression of oral movements.

MATERIALS AND METHODS

12 Male Sprague–Dawley rats weighing 350 – 400 g were used. The rats were anaesthetized with sodium pentobarbitone (40 mg/kg i.p.) and placed in a stereotactic frame. Following surgical exposure, PHA–L was unilaterally injected into either the dmS or the vlS, and CTB was injected into the pcRF. The injections (0.05 – 0.1 μl) were made with glass micropipettes (tip diameter, 20 – 30 μm) attached to a 1 μl syringe filled with either 2.5 % PHA–L (Vector Lab., Burlingame, CA) or 1 % CTB (Vector Lab., Burlingame, CA) in 0.01 M sodium phosphate–buffered saline (PBS, pH 8.0 for PHA–L and pH 7.3 for CTB, respectively). The coordinates for the dmS and vlS were based on our above–mentioned pharmacological studies (4,5,12), i.e. dmS, anterior = 8.6 ~ 10.0, lateral = 2.0 ~ 3.5, vertical = 5.0 ~ 6.5; vlS, anterior = 7.2 ~ 9.7, lateral = 3.0 ~ 4.5, vertical = 3.0 ~ 4.5; and the pcRF, anterior = –0.2 ~ –0.8, lateral = 1.0 ~ 2.0, vertical = 1.0 ~ 2.5 (16). After 14 days of survival, the rats were given an overdose of sodium pentobarbitone (80 mg/kg i.p.) and subsequently transcardially perfused with PBS (pH 7.4) followed by 4 % paraformaldehyde in 0.1 M phosphate buffer. Serial coronal sections (50 μm) were cut and processed with PHA–L and CTB immunohistochemistry using the ABC (Vector Lab., Burlingame, CA) staining technique according to previously described procedures (17). Goat anti–PHA–L (1 : 2000; Vector Lab., Burlingame, CA) was used as the primary anti–serum to PHA–L, and when double staining with PHA–L and CTB

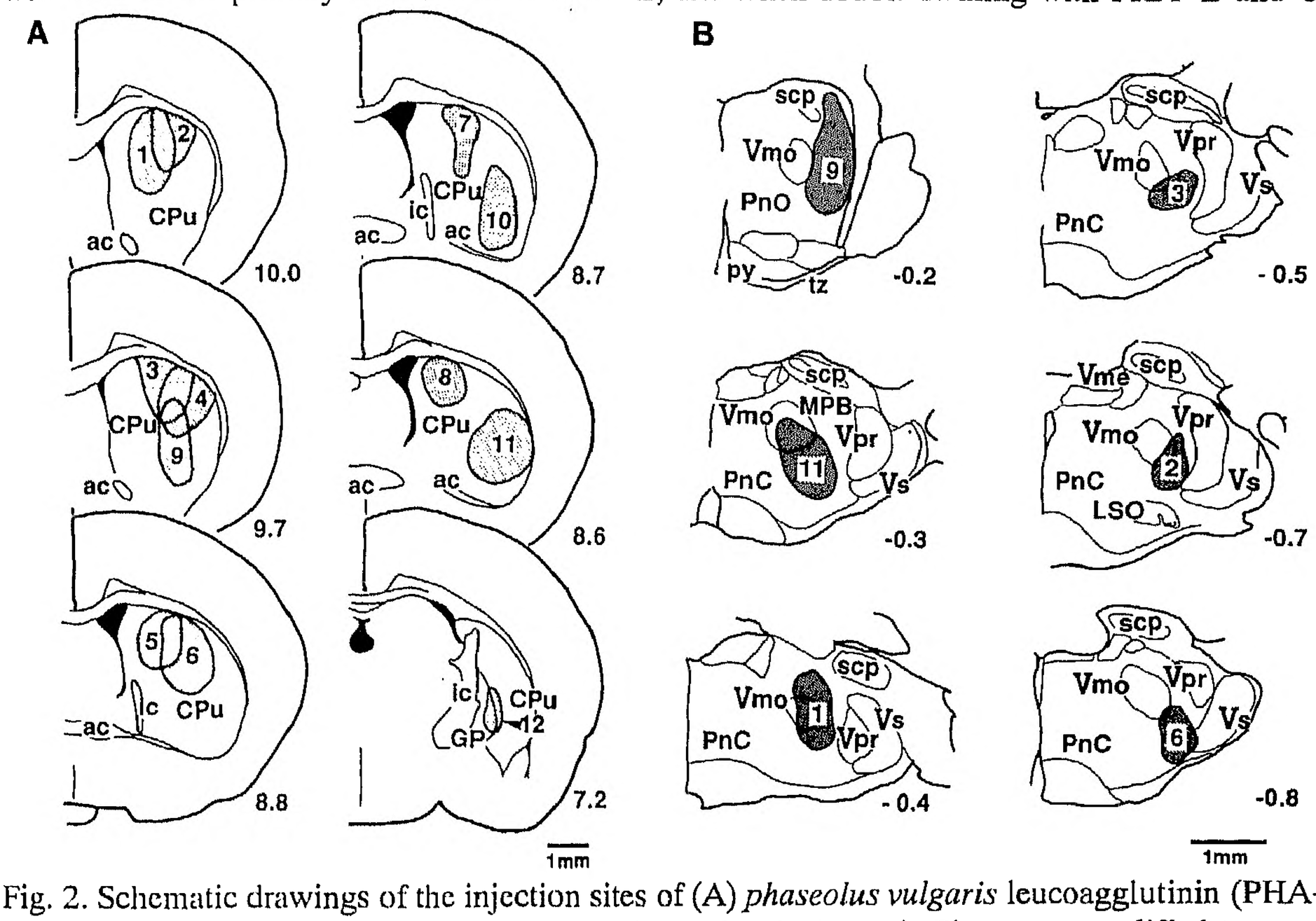

Fig. 2. Schematic drawings of the injection sites of (A) *phaseolus vulgaris* leucoagglutinin (PHA–L; shaded areas) and (B) cholcratoxin subunit B (CTB; hatched areas). Planes are modified to a series of two or three sections for each injection from the atlas of Paxinos and Watson (16); approximate coordinates are indicated in mm anterior to the interaural line. ac: anterior commissure; CPu: caudate putamen; GP: globus pallidus; ic: internal capsule; LSO: lateral superior olive; Vme: mesencephalic trigeminal nucleus; MPB: medial parabrachial nucleus; PnC: pontine reticular nucleus, caudal part; PnO: pontine reticular nucleus, oral part; Vpr: principal sensory trigeminal nucleus; py: pyramidal tract; Vs: sensory root trigeminal nerve; scp: superior cerebellar peduncle; tz: trapezoid body.

was assessed, goat–anti–CTB (1 : 10,000; List Biol. Lab., Campbell, CA) was applied to the same sections instead of goat anti–PHA–L. Alternate sections were counterstained with thionin to identify the nuclei. Labelled axons and varicosities were drawn with the aid of a camera lucida drawing tube.

RESULTS

The PHA–L immunoreactivity was primarily found in the dmS of 8 rats (fig. 2A: no. 1–8), of which 4 rats received an adequate injection of CTB into the pcRF (fig. 2B: no. 1–3 and 6), and the vlS of 4 rats (fig. 2A: no. 9–12), of which 2 rats (no. 9 and 11) received an injection of CTB into the pcRF. The PHA–L immunoreactivity in the dmS was primarily limited to this region in all 8 rats, whereas that in the vlS extended to the globus pallidus in one rat (no. 12) and the middle region of the striatum in another rat (no. 9). The CTB injection sites extended both rostrally and caudally within the pcRF around Vmo and the diffusion halo included adjacent structures such as the principal sensory trigeminal nucleus. The labelling pattern of a representative example of each experiment is described below.

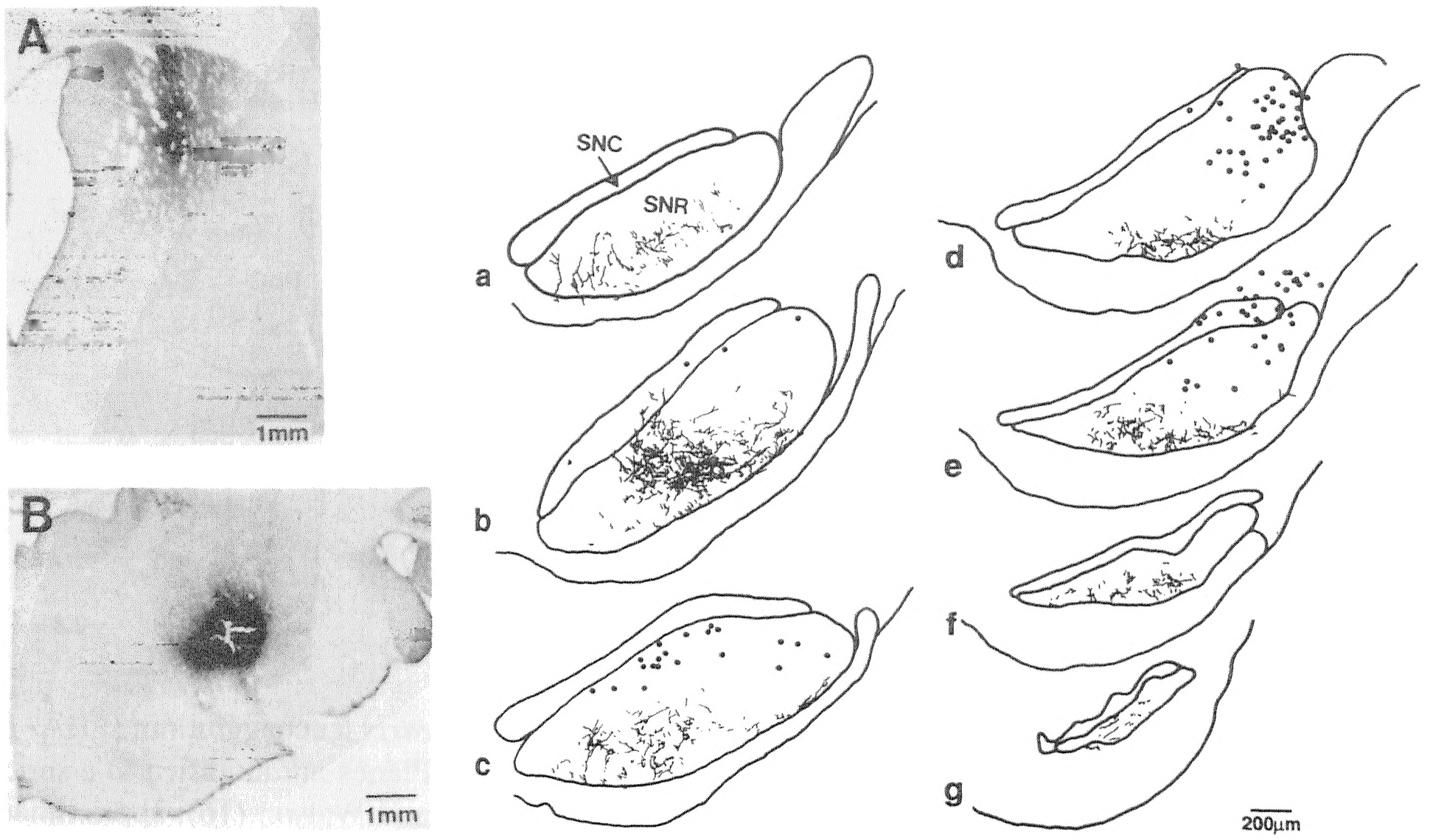

Fig. 3. Low–power photomicrographs of PHA–L injection sites in the dmS (A) and CTB (B) in the pcRF around the Vmo (rat no. 3), and camera lucida drawings of PHA–L positive fibres and plots of CTB positive cells at rostral (a) to caudal (g) levels through the SNR. CTB positive cells without axonal contacts from the anterogradely labelled striatonigral fibres are illustrated by dots.

1. Injection of PHA–L into the dmS and of CTB into the pcRF: rat no. 3. The injection of PHA–L (fig. 3A) resulted in the anterograde labelling of varicose fibres in the ipsilateral globus pallidus and SNR. There was no labelling in the ipsilateral vlS. Labelled axons of neurons in the dmS were running into a posterolateral direction, penetrated the cerebral peduncle and the internal capsule (ic), and reached the SNR. Labelling in the SNR was restricted to its middle and ventrolateral part (vlSNR; fig. 3b–e) and extended from caudal to rostral levels (fig. 3a,f,g). A similar labelling pattern was seen in rats no. 1–2 and 4–8. Following CTB injection into the pcRF around Vmo (fig. 3B), CTB positive cells were found in the dorsomedial and dorsolateral part of the SNR (fig. 3b–e). No contacts were found between PHA–L positive varicosities and CTB positive cells in the vlSNR. A similar labelling pattern was seen in rats no. 1, 2 and 6.

2. Injection of PHA–L into the vlS and of CTB into the pcRF: rat no. 11. Following PHA–L injection into the vlS (fig. 4A), dense labelling was observed in the ic and SNR. The labelled

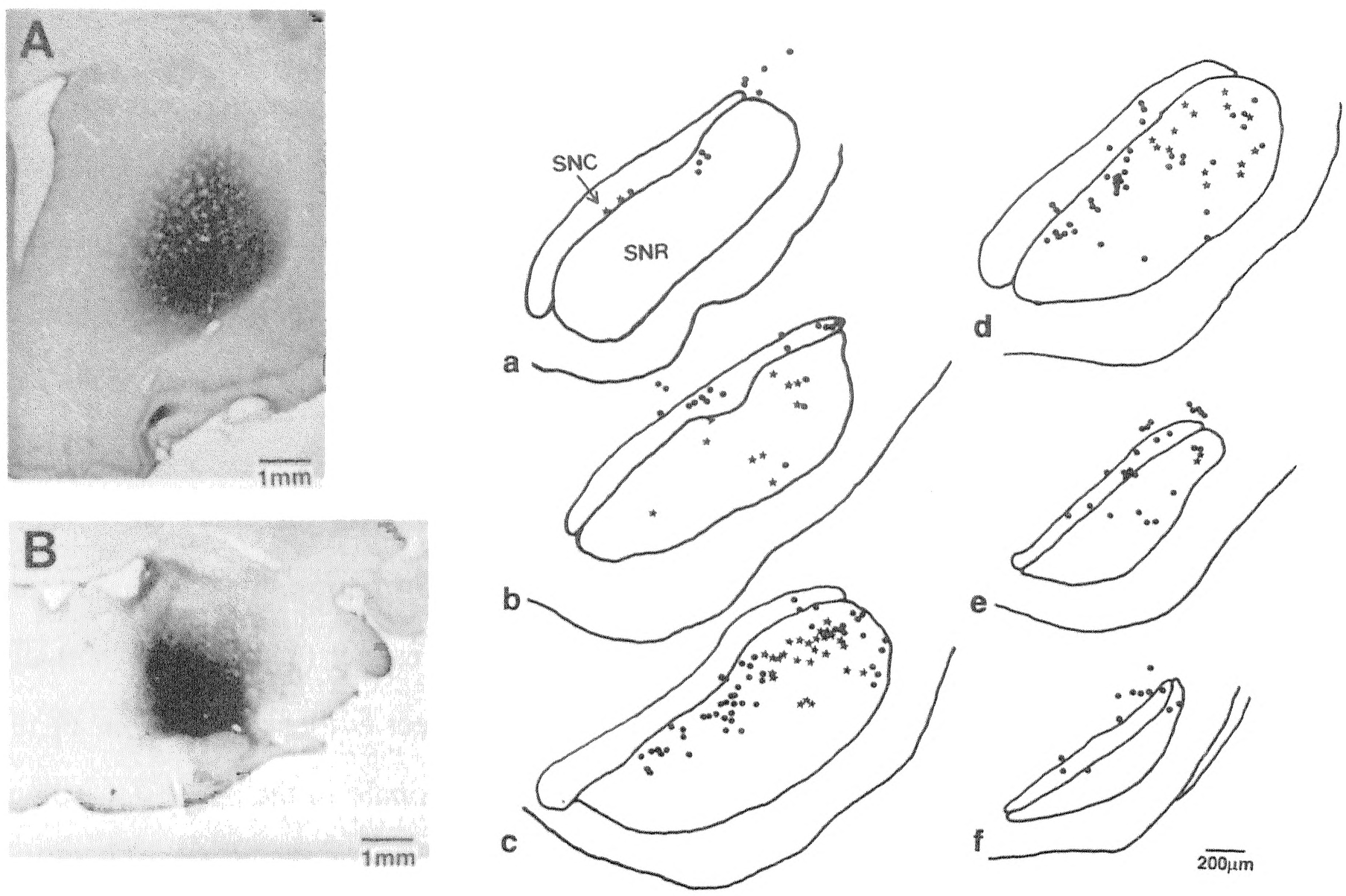

Fig. 4. Low–power photomicrographs of PHA–L injection sites in the vlS (A) and CTB (B) in the pcRF around the Vmo (rat no. 11), and distribution of CTB positive cells with axonal contacts from the vlS (stars) and those without contact (dots) at rostral (a) to caudal (f) levels through the SNR. Note that CTB positive cells with axonal contacts are mainly distributed in the dorsal half of the SNR.

axons were running ventrolaterally from the vlS, penetrated the ic and the middle portion of the substantia nigra pars compacta, and terminated predominantly in the dlSNR. A similar labelling pattern was seen in rats no. 9, 10 and 12. Following CTB injection into the pcRF around Vmo (fig. 4B), CTB positive cells were found in the dorsomedial and dorsolateral part of the SNR. Figure 4a–f illustrates the distribution of CTB positive cells in the SNR with and without apparent contact by anterogradely labelled PHA–L immunoreactive varicosities. The dlSNR contained both a large number of PHA–L anterogradely labelled fibres with varicosities and a large number of CTB positive retrogradely labelled cells. Many CTB positive cells were surrounded by PHA–L positive varicosities (fig. 5A, B and C). Numerous cells of this type were found predominantly in the dlSNR, but some of these CTB positive cells missed contact with PHA–L positive varicosities (fig. 5D). Most of the contacts between PHA–L positive varicosities and CTB positive cells in the dlSNR were located on the soma and proximal dendrites. A similar labelling pattern was seen in rat no. 9.

DISCUSSION

The present study was guided by the outcome of the pharmacological studies performed in the same laboratory (4,5,12). This approach had the advantage that the anatomical experiments could be matched with the earlier reported pharmacological experiments in all aspects. Apart from using the same type of techniques, methods and animals, we purposely directed the PHA–L injections at striatal regions which have been found to have a differential effect upon the expression of oral movements, namely the vlS and the dmS as delineated in fig. 1.

In line with earlier studies (1,2,8,10), we found that the vlS primarily projects onto neurons in the dlSNR, a region that, in turn, projects to the medullary reticular formation. Given the fact that our CTB injections into the RF were far smaller than those described earlier (1) and were actually restricted to the pcRF which embeds the Vmo, this study provides direct evidence in favour of the suggestion of von Krosigk et al. (1) that the nigroreticular neurons terminate in the pcRF. Given our data together with the known electron microscopic data about the nature of the contacts between striato–nigral neurons and nigroreticular neurons in the dlSNR (1), it is concluded that there indeed exists a monosynaptic connection between the vlS and the pcRF which contains neurons that are directly connected with the Vmo (3).

Apart from the finding that the dmS primarily projects onto neurons in the vlSNR, a finding

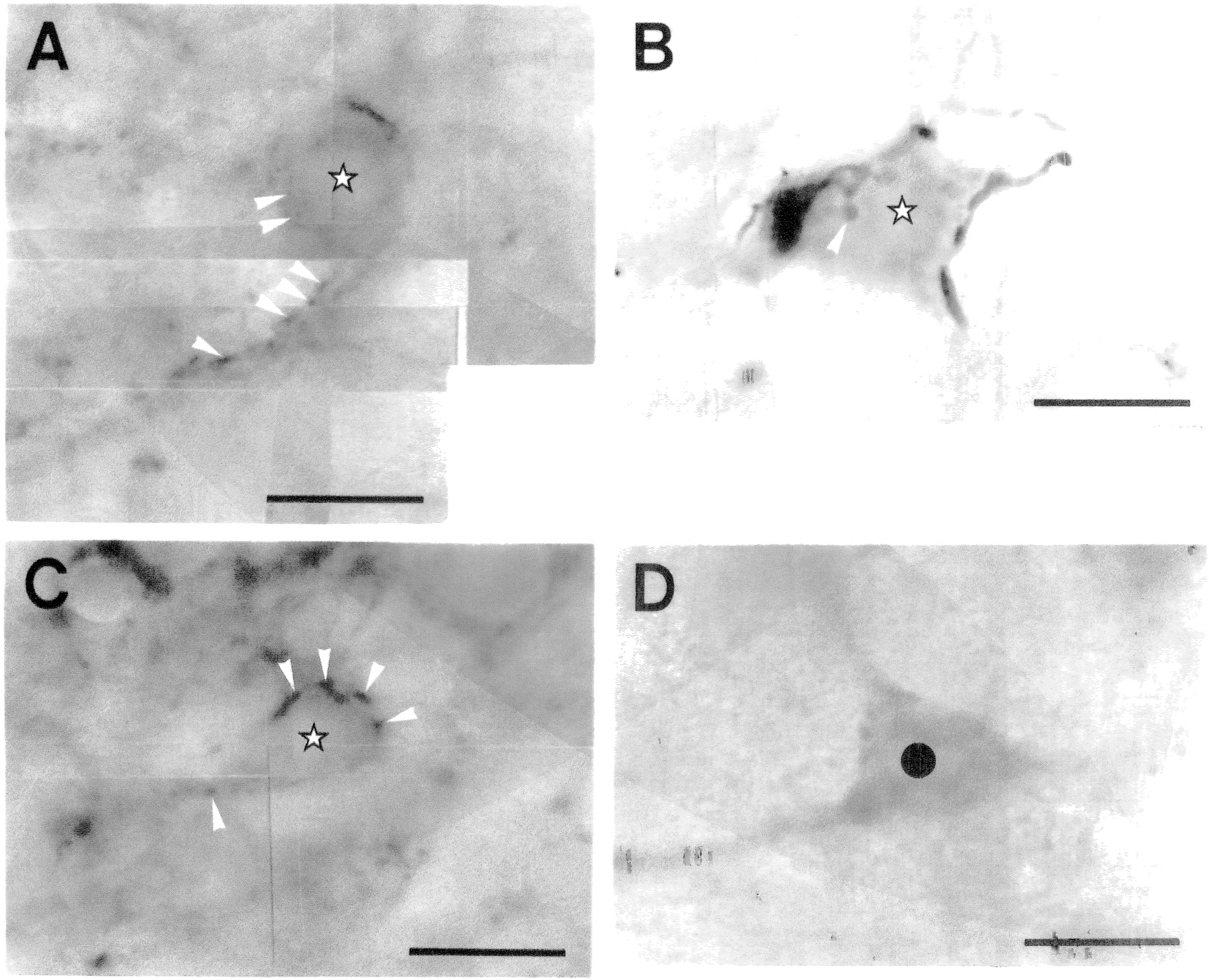

Fig. 5. High-power photomicrographs of CTB positive cells (open stars) with axonal contacts (arrowheads) from the vlS (A, B and C) and that without contacts (dot, D) (rat no. 11). Scale bar = 20 μm.

consistent with earlier reported data (9,11), we found now that this vlSNR does not project to the pcRF: no CTB positive cells could be found in this region following CTB injections into the pcRF. In other words, this study provides evidence that the vlS and dmS are differentially connected with the pcRF.

As mentioned in the Introduction, the vlS plays a critical role in the generation of oral movements, whereas the dmS plays at best a regulatory role in this respect. This study shows that this differential role of the vlS and the dmS in the expression of oral movements can be directly related to their differential connections with the pcRF.

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